

校编码: 10384

分类号\_\_\_\_\_密级\_\_\_\_\_

学号: 24520131153502

UDC\_\_\_\_\_

厦 门 大 学

硕士学位论文

双氢青蒿素对于非小细胞肺癌的作用及其  
机制研究

The Role and Mechanism of Dihydroartemisinin in the  
Development of Non-Small Cell Lung Carcinoma

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论文提交日期: 2016 年 月 日

论文答辩时间: 2016 年 月 日

学位授予日期: 2016 年 月 日

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## 中文摘要

### 背景及目的

肺癌是最常见的恶性肿瘤，在全世界范围内，也是肿瘤导致死亡的首位原因。非小细胞肺癌是肺癌最常见的一种类型。非小细胞肺癌细胞对凋亡的抵抗性是抗肿瘤治疗的一大主要的障碍。因此，目前研究关注的热点是那些能提高治疗抵抗非小细胞肺癌细胞凋亡的新型复合物的发展。双氢青蒿素是一种重要的青蒿素衍生物，是从中草药植物青蒿素中提取出来的天然产物。作为一种非常有效的抗疟疾药物，双氢青蒿素（DHA）在全世界范围内已经是治疗抗疟原虫的一线治疗药。近期研究发现 DHA 对乳腺癌，乳头瘤病毒导致的宫颈癌，肝癌和胰腺癌有着良好的效果，此外，DHA 对肺癌可以通过诱导凋亡达到抗肿瘤的作用，而没有明显的副作用。并且，电离辐射可以使 DHA 诱导非小细胞肺癌细胞凋亡。除了它的突出的凋亡效果，DHA 还会影响肿瘤细胞的功能，包括肿瘤的增殖、血管发生、免疫调节。但是，DHA 抗肿瘤的确切机制仍然需要研究。很多肿瘤细胞有都有一种特征，可以增加葡萄糖摄取和有氧糖酵解。生成乳酸的糖酵解和通过柠檬酸降低线粒体氧化磷酸化代谢（TCA）循环常见于癌细胞。这显著的代谢变化，称为 Warburg 效应，使肿瘤细胞在缺氧条件下同样有优势。因此，肿瘤细胞对于糖酵解的特殊性使得其对于特异糖酵解靶点抑制剂的干扰治疗更敏感。糖酵解抑制剂 2DG，作用于糖酵解起始酶己糖激酶，已经被作为一种有前景的靶向肿瘤代谢改变的治疗化合物研究了。一些证据表明，作用于糖酵解治疗非小细胞肺癌可能是一个很好的方法。这些用糖酵解抑制剂 2-脱氧葡萄糖（2DG）处理的非小细胞肺癌细胞出现线粒体呼吸缺陷，凋亡增多现象。本研究是以两种非小细胞肺癌细胞为研究对象，研究双氢青蒿素及联合 2-脱氧葡萄糖(2DG)对它们的作用及机制研究。

### 实验方法

- 1、MTT 法检测细胞的存活率
- 2、克隆形成实验检测细胞的增殖
- 3、流式细胞术检测细胞凋亡率
- 4、全自动生化分析仪测定培养基葡萄糖含量

- 5、乳酸检测试剂盒检测乳酸的含量
- 6、ATP 检测试剂盒检测 ATP 的含量
- 7、蛋白印记分析细胞蛋白量
- 8、荧光光度计法检测半胱天冬酶的活性
- 9、流式细胞术测定细胞内活性氧生成量

### 实验结果

- 1、双氢青蒿素能减少非小细胞肺癌细胞存活率
- 2、双氢青蒿素抑制非小细胞肺癌细胞的增殖
- 3、双氢青蒿素诱导非小细胞肺癌细胞的凋亡
- 4、双氢青蒿素抑制非小细胞肺癌细胞对葡萄糖的摄取
- 5、双氢青蒿素抑制非小细胞肺癌细胞的糖酵解代谢
- 6、双氢青蒿素引起的非小细胞肺癌细胞糖酵解代谢与 mTOR 激活和 GLUT1 表达受到抑制有关
- 7、上调 mTOR 活化程度能增加被双氢青蒿素抑制的糖代谢水平及细胞的存活率
- 8、葡萄糖转运蛋白 1 的过表达可抑制双氢青蒿素引起的细胞死亡
- 9、葡萄糖干扰双氢青蒿素引起的非小细胞肺癌细胞毒性作用
- 10、双氢青蒿素联合糖酵解抑制剂作用于非小细胞肺癌细胞可以减少细胞的存活率和诱导细胞凋亡
- 11、双氢青蒿素联合 2 脱氧葡萄糖作用于非小细胞肺癌细胞是通过内源性和外源性两个途径来诱导细胞凋亡的。

### 结论

我们的研究证明在 A549 细胞和 PC-9 细胞中，双氢青蒿素可以降低细胞活力及细胞的集落形成的能力，诱导细胞凋亡。此外，我们还首次证明在非小细胞肺癌中 DHA 可以抑制葡萄糖的摄取。而且，DHA 还可以是其的糖酵解代谢减弱，抑制 ATP 及乳酸的生成。因此，我们证明经过 DHA 处理的非小细胞肺癌细胞中的磷酸化的 S6 核糖体蛋白及 MTOR 蛋白还有 GLUT1 蛋白水平明显减少。高表达的 Rherb 作用的 MTOR 蛋白的上调作用可以增加糖酵解代谢和细胞的活力及，而这一作用被 DHA 抑制了。这些结果都证明 DHA 抑制的糖酵解代谢可能和 MTOR 蛋白激活和 GLUT1 蛋白表达有关。我们还发现 GLUT1 蛋白的过表达显著减弱了

DHA 介导的非小细胞肺癌细胞的凋亡。在 A549 及 PC-9 细胞的实验中，DHA 联合 2-脱氧-D 葡萄糖（一种糖酵解抑制剂）显著减少了细胞的活力，增加了细胞的凋亡。然而，这两种物质的联合使用对 WI-38 细胞（一种正常的肺纤维细胞系）的毒性作用非常微小。更重要的，2DG 协同 DHA 诱导半胱氨酸酶-9,8,3 的激活，同时提高了细胞质细胞色素酶 c 和 AIF 的水平。然而，2DG 不能提高由 DHA 引起的活性氧的水平。总之，以上结果显示，DHA 联合 2DG 在非小细胞肺癌细胞诱导凋亡作用同时包含体内固有和外源凋亡途径。生成乳酸和通过三羧酸循环减少的线粒体有氧磷酸化代谢常见于肿瘤细胞中。

**关键词：**双氢青蒿素；细胞凋亡；非小细胞肺癌细胞

## Abstract

### Background and Objective

Lung cancer is the most common malignant tumor and the leading cause of cancer-related mortality worldwide. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. Resistance of NSCLC cells to apoptosis is a major obstacle in anticancer treatment. Accordingly, current researches focus on the development of innovative compounds that promote the apoptosis of therapy-resistant NSCLC cells. Dihydroartemisinin (DHA) is an important derivative of Artemisinin, a natural product isolated from Chinese medicinal herb *Artemisia annua* L. (qinghao). As a very potent anti-malarial drug, DHA has been used as first-line therapeutics against malaria *falciparum* worldwide. Recently, studies have shown that DHA has profound effect against breast cancer, papillomavirus-expressing cervical cancer, liver cancer and pancreatic cancer. Additionally, DHA has been shown to exert anticancer effects by induction of apoptosis without obvious side effects in lung carcinomas. Moreover, ionizing radiation potentiates DHA-induced NSCLC cells apoptosis. Apart from its prominent pro-apoptotic effect, DHA affects cancer cell functions, including tumor cell proliferation, angiogenesis, and immune regulation. However, the exact molecular mechanisms of DHA anticancer effects remain to be fully investigated. This remarkable metabolic reprogramming, known as the Warburg effect, provides cancer cells an advantage to grow even in regions with hypoxia. Therefore, the especial dependence of cancer cells on glycolysis makes them vulnerable to therapeutic intervention with specific glycolysis target inhibitors. The glycolytic inhibitor 2-Deoxy-D-glucose (2DG), targeting hexokinase which is the entry-point enzyme for glycolysis, has been studied as a promising therapeutic compound that targets metabolic alterations of tumor cells. Some pieces of evidences suggest that targeting glycolysis could be a good strategy against NSCLC. These NSCLC cells treated with glycolysis inhibitor 2DG display mitochondrial

respiratory defects and increased apoptosis. We took Non small cell lung cancer cell A549, PC-9 as our research subjects and observed the role and mechanism of Dihydroartemisinin in the Development of Non-Small Cell Lung Carcinoma.

### **Experimental methods**

1. Measurements of cell viability by MTT
2. Measurements of hyperplasia by clonogenic cell survival
3. Measurements of apoptosis rate by flow cytometry
4. Measurements of glucose levels by automatic analyzer
5. Measurements of lactate production of cells in the media by Lactate Assay Kit
6. Measurements of Cell ATP content by ATP Assay Kit
7. Western blot analysis protein
8. Measurements of Caspase activity by VersaFluor Fluorometer
9. Measurement of intracellular ROS generation by flow cytometry

### **Experimental results**

1. DHA inhibits cell viability in NSCLC cells
2. DHA inhibits cell colony formation in NSCLC cells
3. DHA induces apoptosis in NSCLC cells
4. DHA decreases glucose uptake in NSCLC cells
5. DHA decreases the level of glycolytic metabolism in NSCLC cells
6. DHA-suppressed glycolytic metabolism is associated with inhibition of mTOR activation and GLUT1 expression
7. Upregulated mTOR activation increases the level of glycolytic metabolism and cell viability inhibited by DHA
8. Overexpression of GLUT1 inhibits cell death triggered by DHA
9. Glucose prevents DHA-induced cytotoxicity in NSCLC cells
10. DHA combined with the glycolysis inhibitor decreases cell viability and induces apoptosis in NSCLC cells
11. DHA plus 2DG induces apoptosis via both extrinsic and intrinsic apoptosis pathways in NSCLC cells

### **Conclusions**

Our study showed that DHA decreased cell viability and colony formation, induced apoptosis in A549 and PC-9 cells. Additionally, we first revealed DHA inhibited glucose uptake in NSCLC cells. Moreover, glycolytic metabolism was attenuated by DHA, including inhibition of ATP and lactate production. Consequently, we demonstrated that the phosphorylated forms of both S6 ribosomal protein and mechanistic target of rapamycin (mTOR), and GLUT1 levels were abrogated by DHA treatment in NSCLC cells. Furthermore, the upregulation of mTOR activation by high expressed Rheb increased the level of glycolytic metabolism and cell viability inhibited by DHA. These results suggested that DHA-suppressed glycolytic metabolism might be associated with mTOR activation and GLUT1 expression. Besides, we showed GLUT1 overexpression significantly attenuated DHA-triggered NSCLC cells apoptosis. Notably, DHA synergized with 2-Deoxy-D-glucose (2DG, a glycolysis inhibitor) to reduce cell viability and increase cell apoptosis in A549 and PC-9 cells. However, the combination of the two compounds displayed minimal toxicity to WI-38 cells, a normal lung fibroblast cell line. More importantly, 2DG synergistically potentiated DHA-induced activation of caspase-9, -8 and -3, as well as the levels of both cytochrome c and AIF of cytoplasm. However, 2DG failed to increase the reactive oxygen species (ROS) level elicited by DHA. Overall, the data shown above indicated DHA plus 2DG induced apoptosis was involved in both extrinsic and intrinsic apoptosis pathways in NSCLC cells.

**Keywords:** Dihydroartemisinin; cell apoptosis; NSCLC



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